

**Recovery Plan**  
**For**  
***Cucumber green mottle mosaic virus***

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# Recovery Plan

## For

### *Cucumber green mottle mosaic virus*

#### I. Introduction

*Cucumber green mottle mosaic virus* (CGMMV) is a very damaging plant virus in many cucurbit growing areas of the world. All cucurbits (Table 1) are susceptible, although some are more tolerant than others. CGMMV causes serious yield losses by directly affecting plant and fruit quality (Fig. 1), and because it is seedborne in many cucurbits CGMMV also can affect cucurbit seed trade by precluding export of cucurbit seeds produced from areas where CGMMV is present to areas that are CGMMV free. The seedborne nature of CGMMV has most likely been responsible for spread of CGMMV to new areas, but once it is introduced CGMMV has the potential to stay.

CGMMV was first reported from the United Kingdom in 1935 (1). Since its original description, it has been reported from several other regions of the world, mostly Europe, Asia and the Middle East (1-7), and is now considered endemic in some of these areas. However, in recent years CGMMV has emerged as a problem in cucurbit production areas where it was previously unknown, and it has caused severe economic losses. For example, it was first reported from Israel in 1990, but since 2007 CGMMV has become more common and caused losses in several additional cucurbit production areas within Israel (8). In 2013 CGMMV was reported from North America, from both the United States and Canada (9, 10). In the U. S. CGMMV was found in California, first in seed production fields in Yolo County, but in 2014 it was found farther south in commercial seedless watermelon production fields. Further research showed that these represented two separate introductions as genetic analysis of the 2013 and 2014 isolates showed them to be distinct (11). The CGMMV found in Alberta, Canada in 2013 was in mini-cucumbers produced in commercial greenhouses (9). The California 2014 and Canada 2013 CGMMV isolates are very similar in nucleotide sequence, suggesting the possibility that they may have originated from the same geographic region. Simultaneous with the reports from North America, CGMMV was reported in the northern territory of Australia for the first time in 2014 in commercial watermelons (12), and in 2015 in watermelons in Queensland. It was subsequently found in other cucurbits and some indigenous weeds. Initial attempts were made to eradicate CGMMV from the northern territory, but in 2015 the decision was made that eradication was no longer possible (12). They are now focused on strategies to manage the problem but eradication efforts continue in Queensland.

CGMMV is an economically important pathogen in part due to the increased globalization of the seed industry. Since CGMMV can be transmitted via cucurbit seeds (13), and cucurbit seeds are produced in many regions of the world, including those where CGMMV has long been established. The seeds can be distributed to other cucurbit producing areas, thus world-wide

dissemination of the virus is highly probable. This has been borne out by the recent outbreaks in California, and dictates that more efforts be made now to understand CGMMV epidemiology and attempt to develop effective management strategies. Here we give information on what is known about CGMMV and related viruses, and what is known about strategies for managing diseases caused by CGMMV.

## **II. CGMMV incidence and relationships to other tobamoviruses.**

CGMMV is a species of the genus *Tobamovirus*, family *Virgiviridae*. The genus *Tobamovirus* contains 35 virus species at this time, and many of these have numerous strains (<http://www.ictvonline.org/virustaxonomy.asp>). New tobamoviruses are continually being discovered (14), thus we can expect the number of species and strains to continue to increase in number.

Many tobamoviruses are important plant pathogens in various crop plants around the world. Some, like *Tobacco mosaic virus*, occur worldwide and disease losses in tobacco (*Nicotiana tabacum*) and tomatoes (*Solanum lycopersicum*) can be high. Fortunately, CGMMV has been more limited in its incidence and distribution. CGMMV has been commonly reported from parts of Eastern Europe and Asia, and more recently from regions within Africa and parts of South America (1-4, 6, 9, 10, 12). There is a report describing CGMMV from samples collected in Antarctica, however, the identification was made only with serological techniques (15). Within the past 3 years CGMMV has been reported in North America and Australia (9, 10, 12). Thus, CGMMV has now been reported from all cucurbit producing regions of the world (8).

CGMMV was the first described, but now four additional tobamovirus species are known to infect cucurbits (1). Of these, CGMMV is the best studied, the most widespread and the most economically important (7). These other cucurbit viruses are more limited in geographic incidence and reported host species. *Kyuri green mottle mosaic virus* (KGMMV), was reported from cucumbers (*Cucumis sativus*) in Japan and originally described as a variant of CGMMV (CGMMV-C; (16)). *Cucumber fruit mottle virus* (CFMMV; (17)) also from cucumbers was reported from Israel, and *Zucchini green mottle mosaic virus* (ZGMMV; (Yoon, 2002 (25)mos) from squash (*Cucurbita pepo*) in Korea. *Cucumber mottle mosaic virus* (CuMoV) was most recently described in 2007 (18) from cucumbers in Japan, but it is likely that additional cucurbit-infecting tobamoviruses will be discovered in the coming years. The members of these five species exhibit many similar properties, most importantly having a host range largely limited to cucurbits and causing similar symptoms on infected plants. This can lead to incorrect identification unless proper diagnostic approaches are used. Fortunately, at this time there are 43 complete CGMMV sequences in GenBank, 13 for KGMMV, three for ZGMMV, five for CFMMV, and two for CuMoV. Comparison of tobamovirus nucleotide sequences shows that the various CGMMV isolates share greater than 89% homology with each other, but only ~ 60% homology with the other cucurbit-infecting tobamoviruses, and less than 50% homology with the non-cucurbit-infecting tobamoviruses (7, 18). Thus, as is explained in greater detail below, nucleotide sequence-based analyses can be used for highly accurate virus identification.

Many plant diseases can be managed by host plant genetic resistance, and there are effective sources of genetic resistance in germplasm repositories, which are used to manage diseases caused by some tobamoviruses. For example, *N* gene-mediated resistance gives specific and effective resistance against *Tobacco mosaic virus* (19) in tobacco, and *Tm-2(2)* resistance is effective for *Tomato mosaic virus* in tomatoes (20). Both have shown very good efficacy for many years and continue to be used commercially in their respective crop plants. However, for many other tobamoviruses, including CGMMV, effective resistance sources are not yet known. There are some commercially available cucumber cultivars with reported resistance to CGMMV, but the resistance is manifested by lower virus titer. Thus these plants still become infected, can show disease symptoms, and can be inoculum sources for subsequent CGMMV spread. Although no plant-derived durable resistance genes for CGMMV are known, transgenic resistance sources of CGMMV and *Cucumber fruit mottle mosaic virus* have been developed and shown to be effective under experimental conditions (21, 22). So far, no transgenic CGMMV resistance has been used commercially.

### **III. CGMMV epidemiology.**

Like all tobamoviruses, CGMMV (and the other cucurbit-infecting tobamoviruses) differ from most other plant viruses as they are not spread plant-to-plant by specific insect or nematode vectors. All tobamoviruses are spread by mechanical wounding of plants. The virions (virus particles) which contain the infectious, genomic RNA are distinct rigid rod-shaped particles of 18 X 300 nm (Fig. 2). Shorter particles also are common, these contain non-infectious CGMMV subgenomic RNAs. The infectious virions are very stable, they can survive on plant pruning equipment, clothing, hands and machinery (8, 23, 24), and are very easily spread by agricultural practices and mechanical means. Tobamoviruses such as CGMMV also can survive in irrigation water, in recirculated greenhouse water, and in soils as the debris from virus-infected plants, all of which can serve as effective inoculum sources under certain conditions (8, 23, 25). CGMMV also has been reported to be in pollen from CGMMV-infected cucurbit plants, and pollen could possibly be an additional means of spread (26).

CGMMV can be seedborne in cucurbits (13). Other tobamoviruses, including many that infect solanaceous plants such as tobacco, tomatoes and peppers are commonly seedborne (14, 23), and for these, the seedborne virus has proven to be a critical source of primary inoculum for subsequent disease development. Because CGMMV can be seed-transmitted in many cucurbits, and cucurbit seeds are produced and then shipped worldwide, the potential for CGMMV introductions into new areas is a real threat. Seedborne CGMMV most likely served as the source for the recent introductions of CGMMV into both North America and Australia. Thus, the seedborne nature of CGMMV in cucurbits is an important aspect of CGMMV epidemiology and likely serves as the most important source of primary inoculum, but this also suggests that CGMMV seed transmission is a key target for developing effective strategies to help control CGMMV in cucurbits.

Controlling primary inoculum and pathogen spread is a more effective disease management strategy than is attempting to control secondary spread for most plant-infecting viruses. Then, if seedborne inoculum is the important primary inoculum source, approaches such as seed treatments to eliminate infectious virus from seeds, or using seed indexing approaches to ensure that seeds harboring infectious virus are not planted could prevent inoculum introduction and help give effective disease control. These strategies have been shown to work very well for many plant viruses, including some tobamoviruses. For some tobamoviruses in tomatoes and peppers seed treatments have been used to eliminate infectious virus from seeds. *Tomato mosaic virus* is carried on the tomato seed exterior surface. Treating seeds with compounds including tri-sodium phosphate and sodium hypochlorite, which can denature infectious virus without negatively impacting seed viability, have shown to be effective (27). But some viruses are carried inside the seedcoat or within the embryo, and then seed treatments are less likely to be effective. However, even if infectious virus cannot be eliminated from seeds, seed indexing to assess the amount of virus in seeds can sometimes be used in disease management strategies. For example, the *Potyvirus*, *Lettuce mosaic virus* (LMV), is seedborne in lettuce (*Lactuca sativa*) seeds (28), it is carried within the embryo. Seed indexing and epidemiological studies have shown that lettuce seeds do not have to be absolutely LMV free for effective disease control, but they must only have seedborne inoculum levels below a threshold (1/30,000 seeds). If so, then the amount of primary inoculum is so low that economically important disease will not develop (29).

For CGMMV, however, so far neither of the above strategies has proven to be effective. CGMMV is carried both on and within the seedcoat, and thus seed treatments such as those used for tomato seeds are not completely effective at eliminating infectious virus (13). Thermal inactivation by heating seeds to denature CGMMV has been attempted but so far has not proven to be consistently effective (13). Similarly, attempts to identify an acceptable threshold of seedborne CGMMV for cucurbits, similar to the strategy used for LMV, have proven to be problematic. This is due in part because cucurbit seeds often contain infectious CGMMV, but the virus is not transmitted to developing seedlings. Presently it is not known exactly how CGMMV is transmitted to germinating seedlings. In general, if seeds are harvested from CGMMV-infected plants and indirect tests such as ELISA and/or RT-PCR are used to test them, these seeds can show 100% positive for CGMMV. However, the rate of seedling infection (seed transmission) after sowing a cohort of these same seeds is typically only in the 1-5% range, or less, under greenhouse conditions. However, high seed transmission rates (76%) from CGMMV-infected cucumber plants have been reported (26).

Serological tests such as ELISA and molecular tests such as RT-PCR detect the presence of virus proteins and RNA, respectively, and their detection does not guarantee that the CGMMV is infectious. But biological assays including inoculating seed extracts to indicator plant show that seeds can contain infectious CGMMV but still fail to transmit it to germinating seedlings. Thus, even if infectious CGMMV is present in/on seeds, it is still not a guarantee that it will be transmitted to germinating seedlings. These confounding factors remain to be understood and

as a result it has so far not been possible to test cucurbit seeds and accurately identify infectious CGMMV, or to differentiate CGMMV that is merely contaminating seeds from that which will infect the developing seedling. More research is needed to attempt to clarify these issues.

Modern cucurbit agriculture also offers many opportunities for tobamovirus spread (30). Some cucurbits are commonly produced in greenhouses where plants may be routinely handled and pruned, providing ample opportunities for CGMMV and other tobamoviruses to be spread. As an example, consider contemporary seedless watermelon production. Watermelon seedlings are germinated in seedling trays with individual cells 1" square and 2" deep. Trays hold 98, 128, 200, or 288 cells. These plants are physically very close together, allowing for constant rubbing between adjacent plants, providing opportunities for CGMMV to spread. Transplanting and other physical plant handling (e.g. grafting) also provides very good opportunities for tobamovirus spread. Some of these tasks result in very small, quick healing wounds, but these are sufficient to introduce highly infectious plant viruses such as CGMMV, and if transplanting is into soils that are CGMMV-infested, the transplanted seedlings can become infected via small wounds on the roots, allowing the resident inoculum present in the soil to gain entry into the susceptible seedlings (8, 31). The recent outbreaks of CGMMV in Canada (cucumbers), Israel (cucumbers and melons), California (cucumbers, melons, watermelons) and in Australia (watermelons and other cucurbits) all involved greenhouse and/or transplanting activities that likely contributed to the development of the resulting epidemics (8-10, 12).

Transplant nurseries start watermelons for all the growers in an area, and will also ship transplant watermelons to more distant purchasers. In one transplant nursery greenhouse, there can be upwards of 100 trays with 200 cells each. If 1% of the seeds contained CGMMV, and if 1% of those seeds resulted in an active infection, there will be 2 infected plants in that greenhouse. For tobamoviruses like CGMMV this could still be significant. With the amount of handling that occurs in transplant production, the potential for CGMMV to spread between seedlings is very high. One recent study showed that after pruning an infected plant, the next nine plants to be pruned developed infection from transmission by the contaminated pruning shears and workers' hands (23). Further opportunities for spread are moving flats for transplanting, transplanting to the field, deflowering in the field, and field tractor work.

The known plant host range of CGMMV is not as broad as are the host ranges for many other tobamoviruses, but alternate host plants could also serve as inoculum sources. Outside of the Cucurbitaceae, only a relative few host plants are known, but in recent years new plant hosts have been identified. *Chenopodium album* ssp. *amaranticolor* and *Datura stramonium* are common virus indicator plant species that react to infection by some CGMMV strains by producing local lesions on inoculated leaves (Fig. 3). *Nicotiana benthamiana* also is a standard indicator species that reacts by systemic infection (Fig. 3). However, it is important to note that all CGMMV isolates do not cause obvious symptoms in CGMMV-infected *N. benthamiana* plants. The recent CGMMV outbreak in Australia has resulted in studies suggesting that various

weed species may be potentially important alternate hosts for CGMMV. CGMMV has been confirmed in plants of *Amaranthus* spp and *Portulaca* spp, however, at this time the role of alternate host plants in CGMMV epidemiology is unknown. Other plants including *Amaranthus retroflexus* (Red root or American pigweed), *Chenopodium album* (lamb'squarter), *Heliotropium europium* (Helitrope), *Portulaca oleracea* (Pigweed or Portulaca weed), *Solanum nigrum* (Nightshade), and *Cucumis myriocarpus* (paddy melon) are potential CGMMV hosts, and they recommend that these plants be removed from around cucurbit production areas (<http://www.nt.gov.au/d/cgmmv/index.cfm?header=Decontamination%20advice>).

#### **IV. CGMMV-induced symptoms and diseases.**

All commercially-grown cucurbit species can be infected by various strains/isolates of CGMMV (32), and most CGMMV-infected cucurbit plants show disease symptoms. CGMMV causes symptoms on leaves and even fruits of infected plants (Fig. 1), and symptoms can vary depending on the CGMMV isolate/strain, time/growth stage when plants are infected, environmental conditions and the host plant species/cultivar (Fig 1). CGMMV causes foliar symptoms of light green mottling or mosaic patterns on the leaves, may cause blistering on the upper leaf surface, and rarely stunts plant growth. Symptoms on fruit can range from no or very few obvious external symptoms, such as in watermelon, to severe fruit distortion, such as seen in most cucumber cultivars. Infection interferes with sugar accumulation and flavor, and may also cause premature degradation of the pulp, making fruit unmarketable for consumption. Watermelon plants infected with CGMMV also develop necrotic lesions on the peduncle. Not all strains of the virus cause fruit symptoms in all cucurbits. Also of note, CGMMV does not seem to affect seed set or appearance, so contaminated seeds are not visually distinguishable from healthy seeds (8, 13).

In a commercial field or greenhouse production setting, losses from CGMMV can be severe, up to 100%, but losses of 40-80% are more common. Losses can be magnified when practices such as in California abatement programs preclude growing cucurbits for three additional years on fields where CGMMV has been confirmed. One disease management strategy that has proven to be effective in Israel is early visual identification and roguing of infected plants. This can help to control CGMMV secondary spread which can be the most economically significant.

#### **V. CGMMV detection and identification.**

A number of commercial testing kits are available to detect CGMMV in both seeds and plants. The seed industry uses serological tests produced by commercial sources (e.g. PRI, Wageningen UR, Wageningen, Netherlands and Agdia Inc., Elkhart, IN) to test cucurbit seeds for CGMMV. This method is the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), and uses antibodies raised against CGMMV antigen to detect the virus in liquid samples. Since this test detects protein, it does not distinguish between infectious and non-infectious virus. The International Seed Health Initiative for Vegetable Crops standard is to test 2,000 seeds per seedlot, and if a positive is found this suggests that the seeds contain sufficient

CGMMV to not be planted. Because this standard has been used for a number of years but the recent CGMMV outbreaks have still occurred, Australian scientists now test 20% of seeds in small seedlots, or 9,400 cucurbit seeds for large seedlots. However, some levels of serological discrepancy against different isolates of CGMMV have been observed in commercial ELISA kits developed from various sources of antibodies (K.-S., Ling. personal communication). Therefore, care should be taken in selecting a sensitive ELISA kit that is appropriate in detecting that particular CGMMV genotype, particularly in seed health assays. There is also a field Immunostrip® (Agdia Inc.) assay for testing plant leaves. The CGMMV antibodies have been immobilized on a small indicator strip, which is placed in a homogenized tissue sample and sample fluid flows over the indicator stripes by capillary action. Results are seen in about 20 minutes. This method can be used for immediate field identification in production areas and is very user friendly, though should not be mistaken for a seed or plant health assay. Any suspected fields should have samples sent to appropriate testing facilities for confirmation.

Direct tissue blot immunoassay (DTBIA) using monoclonal antibodies has been developed, though DTBIA is rarely used. For this method, plant sap is blotted onto nitrocellulose membranes, then the membrane is incubated with monoclonal antibodies of the target virus. Then, an enzyme-linked secondary antibody is used to detect the primary antibody. This method has similar detection thresholds to DAS-ELISA, but is simpler to use under production conditions (33).

In research labs and for diagnostic confirmation both for the California Department of Food and Agriculture (CDFA) and USDA APHIS-PPQ, molecular techniques based on CGMMV genomic RNA sequences are used to confirm virus presence. CGMMV is a single stranded RNA virus. The genomic RNA template is first reverse-transcribed (RT) into complementary DNA (cDNA), and then the cDNA is amplified in a polymerase chain reaction (PCR). The RT step can be template specific, i.e. primed with CGMMV sequence-specific oligonucleotides, or randomly primed with random hexamer oligonucleotides, to generate cDNA. Either type of cDNA can then be amplified with sequence specific primers to generate many copies of cDNA, which can be analyzed by agarose gel electrophoresis for visual identification of amplicons. These can, also used for Sanger sequencing to confirm the identity of the virus and then compare its relationships to other known CGMMVs. For example, nucleotide sequence and phylogenetic analyses showed that the California 2013 (Yolo) and 2014 (San Joaquin) CGMMV isolates were distinct CGMMV isolates (Fig. 5) and most likely represented two separate introductions. There are several CGMMV sequence-specific primer pairs that are used in diagnostic work, the most common of which were developed by Shang et al. to amplify a region of the CGMMV coat protein gene (33). Quantitative RT-PCR, or real-time RT-PCR assays have also been developed and used in research and for diagnostics, which gives quantitative data of how much amplicon is present in the sample (33).

The above assays work extremely well for diagnosing CGMMV-infected plants, but the most important issues that arise when testing seeds are do these data represent infectious CGMMV,



and if so will it be transmitted to germinating seedlings? DAS-ELISA indicates the presence of viral coat proteins, and RT-PCR shows the presence of the target RNA sequence, but not whether the full RNA molecule is present. The third diagnostic method which could help fill this gap is biological indexing. For this method, suspected contaminated or infected plant parts, including seeds, are homogenized in an inoculation buffer, and then with an abrasive rubbed onto leaves of a plant that gives a rapid and specific reaction to the suspect virus. For CGMMV, *Chenopodium album* ssp. *amaranticolor* responds in approximately 5 days by forming local lesions on the inoculated leaf where infectious virus was introduced (Fig. 3). *Nicotiana benthamiana* rub inoculation results in a systemic infection, but as noted above, not all CGMMV isolates give obvious symptoms on *N. benthamiana* plants, thus this plant species is not a good choice for seed testing. The main drawbacks to biological indexing are the level of sensitivity compared to either molecular or serological techniques, and that it fails to differentiate between CGMMV that is going to be transmitted via seeds to germinating seedlings and that which is still infectious but merely contaminating seeds. Molecular detection methods have been proven to be capable of detecting tobamoviruses down to a concentration of 10-100fg/mL, as compared to ELISA where sensitivity limits can be on the order of 1ng/mL. This represents an increase of sensitivity of  $10^5$  (34). Biological indexing is less sensitive than ELISA, so the method can be used to confirm the presence of infectious virus, but a negative result still must be interpreted with caution.

## **VI. Response**

Currently CGMMV is considered by CDFA as a quarantine actionable “A” rated pathogen. Eradication efforts are overseen by CDFA in California. However, the pathogen classification by USDA APHIS-PPQ did not occur until July 17<sup>th</sup>, 2014. During the initial outbreak in 2013, identification of CGMMV was the first hurdle to disease response. The outbreak occurred in a commercial seed increase block in Yolo County, within a field with three adjacent cucurbit crops. The grower had no prior knowledge of CGMMV, and under field growing conditions the disease appeared to have mild to moderate impact on growth (Fig. 4). At the time, the disease was also unknown to Cooperative Extension personnel, who are usually the first individuals to identify pathogens in the field. During a routine phytosanitary inspection by the county biologists, samples were collected from the field. Subsequently CGMMV was identified by Dr. Tongyan Tian, virology specialist with CDFA. Prior to this CGMMV was not known to occur in the US, and so the USDA APHIS-PPQ lab had to also confirm the identification conducted by CDFA. When the pathogen was identified as CGMMV, quarantine and mitigation efforts were undertaken. The cucurbit crops were destroyed and the field put under an abatement order, allowing only non-host crops to be grown in the field for three years. The total impacted area was less than 10 acres, and there were no other cucurbit crops in the vicinity. To date, eradication efforts for that site seem to have been successful.

The 2014 response was also delayed, with identification and response taking approximately 3-4 weeks after concern was raised by the county farm advisor for the first field. The grower

became concerned in mid-July when fruit harvested from the field was rejected for poor quality and low sugar. This was after the third harvest of seedless watermelons. Dr. Aegerter made a farm call on July 16<sup>th</sup>, at which time she sampled the field. She was notified of positive identification of CGMMV by UC Davis laboratory personnel on July 28<sup>th</sup>, and regulatory sample results were released August 6<sup>th</sup>. In August of that year, two more CGMMV-positive fields in San Joaquin County were identified, and one in Fresno County, and one in Kern County. Each field was eventually put under quarantine orders, the crops destroyed, and a three year abatement enacted.

At the local level, CGMMV outbreak response was slow. It was one calendar year between identification in 2013 and official listing of the pathogen by USDA APHIS-PPQ. This delayed dissemination of information on the disease to in-field responders, UCCE Farm Advisors. When the 2014 outbreak occurred, there was no state or federal response plan to follow, which caused confusion between the impacted growers and UCCE personnel. As the 2014 outbreak progressed, management plans were developed and implemented. Had there been a plan developed prior to the 2014 growing season, the identification and response time would have been faster and more efficient. Due to the larger outbreak of 2014, there is now an outbreak plan, with the following steps: positive identification by a regulatory agency, quarantine of the infected field, crop destruction, and subsequent three years of non-host production.

On a larger scale, the identification of infected seed lots is an ongoing issue. Due to the California outbreaks, growers who purchase cucurbit seeds now demand phytosanitary certificates for seed to be CGMMV tested, and the sample tested to be free of CGMMV. Seed companies that produce and move cucurbit seed internationally have, for the most part, complied and added CGMMV tests to the list of seed health testing. The seed industry is actively participating in research and diagnostic development to control the dissemination of CGMMV. However, it has only been this year, 2016, that mandatory reporting of CGMMV infected seeds to regulatory agencies has been required. Prior to that, it was at the discretion of the individual company or testing facility whether to notify state or federal authorities to the presence of an infected seed lot. Also, there is no formalized disease response plan for the destruction of CGMMV- infected seed lots. There are feasibility issues with destroying a commercial seed lot, which can be upwards 1,000 kg.

## **VII. Mitigation and disease management**

Cucurbit production is not a zero risk endeavor but the benefits outweigh the potential risks so long as those involved are aware and efforts and procedures are in place to minimize potential CGMMV-induced effects. The probability of future CGMMV introductions into the U. S. and disease development in cucurbits must be considered as likely to occur again, as has been seen in Australia. There, the 2016/2017 growing season has seen a new outbreak, this time in greenhouse production systems in Western Australia. Overall, the best method of pathogen control is stringent seed testing requirements and exclusion of contaminated seeds. To prevent CGMMV from becoming a production system-wide problem, an integrative approach including

seed health assays, resistance breeding, and greenhouse nursery surveillance would be necessary.

How can we be proactive in safeguarding our vast cucurbit production from CGMMV infection and minimize its potential adverse effects on yield and economic losses? An integrative approach will be necessary to prevent CGMMV from introduction through strict seed-health tests and early identification of infection in nursery seedling facilities and production fields. Improvement in the sensitivity and reliability in seed health assays is also dependent on the thorough characterization and understanding of genetic diversity of the virus. Effectiveness of seed treatments is also in need of improvement. For long term management of this potentially devastating virus, breeding for disease resistance is in need of identifying genetic sources of resistance in the national germplasm collections. If sources of resistance are unavailable, then biotechnology in developing transgenic plants against CGMMV should be considered. Genetically modified squash cultivars resistant to several common viruses have been successfully developed and are in commercial production. In the meantime, for short term disease management, grafting susceptible cucurbit materials to a CGMMV-resistant rootstock could be a quick solution to break the disease cycle.

The following sections describe mitigation at different points of cucurbit production.

#### Transplant production greenhouse control measures

Transplant greenhouse growers should only use seed that has been tested and found free of evidence of CGMMV. Request copies of the test certificate stating that the seed lot is “CGMMV free”. Although whether the current standard of 2000 seeds tested for CGMMV is adequate remains a question and Australia has already increased the threshold to testing 20% of a seedlot or 9400 seeds for large seedlots.

Virus symptoms are not likely to be expressed strongly in young transplants, thus visual inspections are problematic in terms of accuracy. However, if any symptoms are noted during transplant production, plants should be tested with something such as in-field test immunostrip, or samples can be submitted to diagnostic labs for rapid testing.

Use strict sanitation in greenhouses. If reusing planting trays, they should be steam-sterilized, not recommended are tray cleaning solutions as they do not adequately penetrate crevices. Soil mixes should be sterilized with steam or fumigation. Avoid having workers or equipment touch plants. Anything which touches plants should be disinfected regularly, especially when moving in between greenhouses. Workers’ boots should be sanitized upon entry and exit to greenhouse, such as by using shoe baths or hand-held spray bottles. Hand wash stations with disinfectants proven to be effective against CGMMV should be present at each greenhouse, with handwashing required before entry and after leaving each greenhouse. Keep greenhouses free of weeds. Upmost care should be used when conducting grafting of plants; all work surfaces and tools should be sanitized at regular intervals. In between crops of transplants,

when the greenhouse is empty, growers should sanitize greenhouse floors, walls, and benches using approaches that are proven to inactivate CGMMV.

Research on disinfectants for other tobamoviruses (TMV) has shown that the most effective disinfectants are Nonfat dried milk (NFDM, 20% (wt/vol) plus 0.1% Tween 20), Vikron-S (2% solution, Chemours), Lysol All-purpose cleaner (50%) and 0.6% sodium hypochlorite (24, 27). Sodium hypochlorite, which is recommended for CGMMV contamination in some cases (<http://www.nt.gov.au/d/cgmmv/index.cfm?header=Decontamination%20advice>) should be made by diluting household bleach 1:10, and it should be noted that the half-life of diluted bleach is very short, so the solution should be changed every two hours. Quaternary ammonium compounds, which are commonly used as disinfectants at greenhouses and are effective against bacteria and fungi, have not proved to be effective against tobamoviruses infecting tomatoes (24, 27), and thus whether or not they will be effective against CGMMV is not known. Of the available materials, operations are often reluctant to use bleach due to its potential to cause corrosion. This can be remedied by rinsing with water after the disinfection period. For all disinfection products, follow product instructions to make sure that the product has the appropriate contact time with the target surfaces to achieve disinfection. Note that organic matter can interfere with the activity of disinfectants, especially sodium hypochlorite. Prior to disinfecting, soil and organic material should be removed from the equipment. Quaternary ammonium compounds are deactivated by anionic detergents, including common soaps, so washing off of debris and soil is best done with plain water. For large equipment and tractors, a pressure washer is highly effective. For wheeled vehicles and equipment, a tire bath can be constructed for the vehicles to roll through.

#### Farm level control measures

When transplanting, keep records of plant sources, including seed lots and greenhouses. These so-called “trace-back” records should already be part of a third-party food safety certification program (if a food safety certification program is already in place at the farm).

Control weeds, especially weeds that may border cucurbit production fields. Of greatest concern would be any weeds in the cucurbit family (e.g. wild melon). However, other non-cucurbit weeds can serve as hosts for the virus. Based on reports from Australia (<http://www.nt.gov.au/d/cgmmv/index.cfm?header=Decontamination%20advice>) and elsewhere, weed hosts include *Amaranthus retroflexus* (redroot pigweed), *Solanum nigrum* (black nightshade), *Portulaca spp.* (purslane), *Physalis spp.* (groundcherry) and *Chenopodium album* (common lambsquarters). Control chewing insects such as beetles which are suspected of being able to transmit the pathogen.

Minimize movement of equipment and people between fields. Proper phytosanitary procedures should be followed when entering and leaving a field. Disinfect equipment and boots between fields. Equipment should be washed clean of dirt prior to a disinfection step.

Any hand-held equipment (i.e. pruning shears, harvesting knives, etc.) that causes mechanical wounding of plants should be disinfected between fields.

Train field workers and fruit pickers to report any unusual looking plants or fruit. Scout fields regularly to look for symptoms. Suspicious looking plants should be sent to a lab for diagnosis (a University, state or private lab equipped to test for the virus).

## **VII. Research, education and extension priorities**

CGMMV rapidly emerged in 2013 and 2014 in various regions of the world. This sudden appearance was coincident with severe economic losses to the immediate crop, but also in California to future cucurbit crops due to the implemented 3 year abatements on growing susceptible crops on lands where CGMMV was found. Efforts are needed to minimize the potential for future introductions. This includes additional research to help prevent CGMMV introductions, but also efforts to educate people to be aware of CGMMV, and plans should be in place so new CGMMV introductions can be rapidly recognized, contained and eliminated.

Seed testing first priority. The current ISHI standard is to test 2,000 seeds by ELISA. This may have to be re-evaluated in terms of the recent outbreaks. Australia already has set higher testing standards including testing 20% of seeds for small seedlots, or 9,400 for large seedlots. Comparison of the ISHI standard of 2000 seeds vs. the Australian standards, if 10 CGMMV infected seeds are in a lot of 100,000 seeds, and if only 2,000 seeds are tested, the probability for CGMMV detection is only 18%. Whereas using these same seeds if 9,400 seeds are tested the probability increases to 63%.

There are continuing issues with CGMMV detection. One problem is the disconnection between detectable infectious CGMMV and transmission rates to seedlings. It is still unclear whether this is a result of the lack of opportunity for infection during germination, detection of noninfectious CGMMV, or some as yet unknown interaction between the virus and the host. What we do know is that the virus can be present on seeds, but only results in infection in a small percentage of seedlings. Further research is needed to understand the factors affecting seedling infection rates, which would possibly allow for better control methods for the greenhouse industry. Another issue with seed testing is the difference between serological methods and molecular detection, and the difference in sensitivity between the two methods. One argument is that serological methods (antibody based detection) only prove the presence of the antigen, coat protein, and not the infectivity of the material detected, thereby giving false positive results. Molecular testing is more sensitive and detects the viral RNA, which is necessary for infection to occur, but it may not be intact, infectious RNA detected. Another problem with molecular methods of seed testing is purifying good quality RNA from seed samples that is free of molecular testing inhibitors. Seeds are structures that evolved to resist degradation in a natural environment, and contain many compounds that inhibit molecular testing methods. Improved RNA purification methods research is ongoing. Research is also ongoing to develop an effective testing protocol that involves serological and molecular

detection methods, with the goal being greater confidence in the presence of infectious virus if both viral coat proteins and RNA are detected.

This leads us to the question, if CGMMV is detected, how can first responders act quickly and effectively? Currently, any seed detection of CGMMV must be reported to state and federal authorities. Any seedlots that are found to harbor the virus should be traced, recalled, and destroyed. In the 2013 outbreak the limitations of seed trace back were found, so improved documentation is needed. If an outbreak goes undetected until the plants are in the field, faster positive identification and quarantine implementation should occur. A standard operating procedure in the event of a positive field detection should be drafted at the state or federal level that includes an expected timeline for the implementation of control measures. The 2014 outbreak took extended periods of time, on the order of weeks, between detection and quarantine implementation. Yet, it was also an opportunity to go through the process of developing responses to a field outbreak, and decisions were made on the length of quarantine, area involved, methods of equipment handling, and included grower involvement. Therefore, the blueprint for a response plan is available, and if the response was reduced to days there would be less chance of spreading the virus beyond the impacted field(s), and the grower(s) involved would have less uncertainty about the future of the impacted area. This type of intervention is costly to the growers, and removing as much uncertainty for them would go a long way in maintaining good relations. Effective communication to all impacted shareholders is necessary.

For the US, outreach materials are available for on-farm use with information covering: phytosanitary practices to avoid spreading CGMMV, disease identification and impacts, and guidelines for best farm practices for disease management. The California Seed Trade Association released a fact sheet, which was updated in February 2015, that included information on the pathogen and best practices to avoid introducing it from seed (<http://www.calseed.org/documents/What%20is%20CGMMV.pdf>). The California seed Association has a webpage (<http://www.calseed.org/cgmmv.html>) with further links to the previous fact sheet and information from workshops about the pathogen, and a comprehensive brochure. Pest alerts on CGMMV have also been released by various states and plant disease networks. The dissemination of information on outbreaks and what to do in the event of an outbreak is extremely important. Often, growers are unfamiliar with new disease symptoms and unsure what steps to take. The people who are most likely to notice disease symptoms in a field or greenhouse setting, farm workers, fruit pickers, and tractor drivers, often do not speak English. Spanish versions of the Seed Trade Association are available. It is important that outreach materials are kept up to date and easily accessible.

Table 1. Scientific and common names of *Cucumber green mottle mosaic virus*-susceptible cucurbit species.

<i>Cucumis anguria</i>	Burr cucumber, burr gherkin
<i>Cucumis melo</i>	Melon
<i>Cucumis sativus</i>	Cucumber
<i>Lagenaria siceraria</i>	Bottle gourd and long melon
<i>Momordica charantia</i>	Bitter gourd
<i>Cucurbita moschata</i>	Butternut squash, Kent pumpkins and Asian gramme
<i>Cucurbita pepo</i>	Zucchini and button squash
<i>Cucurbita maxima</i>	squash
<i>Luffa acutangula</i>	Angled luffa, Sinqvar
<i>Benincasa hispida</i>	Winter or hairy melon
<i>Cucumis metuliferus</i>	Horned melon or kiwano or African horned cucumber
<i>Citrullus colocynthis</i>	Bitter paddy melon
<i>Citrullus lanatus</i>	Wild melon
<i>Cucumis myriocarpus</i>	Prickly paddy melon
<i>Luffa cylindrica</i>	Smooth luffa
<i>Trichosanthes cucumerina</i>	Snake gourd

These data are from: [http://www.nt.gov.au/d/cgmmv/news\\_media\\_archive\\_article.cfm?newsid=563&ws=1](http://www.nt.gov.au/d/cgmmv/news_media_archive_article.cfm?newsid=563&ws=1) ;  
[http://www.nt.gov.au/ntg/gazette/2015/docs/S22\\_2015.pdf](http://www.nt.gov.au/ntg/gazette/2015/docs/S22_2015.pdf)

Figures:



Figure 1. CGMMV symptoms. Upper left, symptomatic fruit at harvest from a commercial field in 2014. Upper right, foliar symptoms in a greenhouse grown watermelon. Lower left, foliar symptoms of cantaloupe. Lower right, foliar symptoms of cucumber. All plants were infected with the same isolate of CGMMV from the 2014 California outbreak. Images courtesy of Tera Pitman, UC Davis.



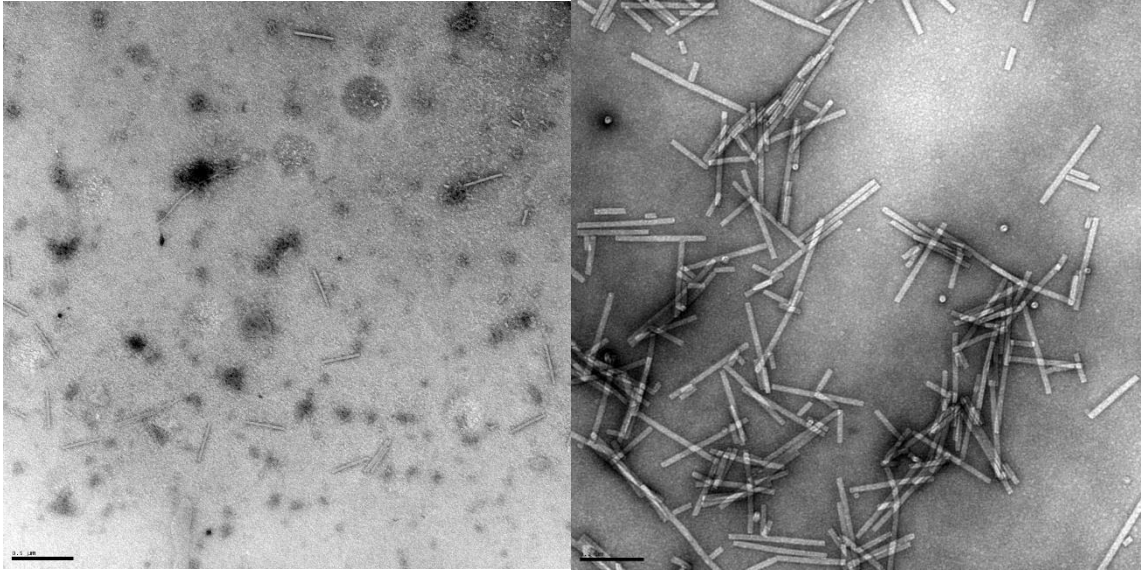


Figure 2. Transmission electron micrograph of (left) sap from field collected symptomatic watermelon showing both flexuous rods and rigid rod structures, scale bar 0.5 $\mu$ m. Nucleic acid analysis indicated co-infection with CGMMV and Papaya ringspot virus. Right, purified CGMMV virions, scale bar 0.1 $\mu$ m. Images courtesy of Dr. Tongyan Tian, CDFA.



Figure 3. Left, local lesions on a *Chenopodium album* ssp. *amaranticolor* leaf 5 days after rub inoculation. Right, CGMMV infected *Nicotiana benthamiana* 10 days post inoculation, showing characteristic leaf distortion and chlorosis. Images courtesy of Tera Pitman, UC Davis.





Figure 4. Commercial watermelon field in San Joaquin County infected with CGMMV. This site was positively identified as infected with CGMMV after three harvests of seedless watermelon, at which time the infection rate was at or near 100%. The grower called UCCE Farm Advisor Dr. Brenna Aegerter when the crop was rejected at the cutting facility for poor quality.

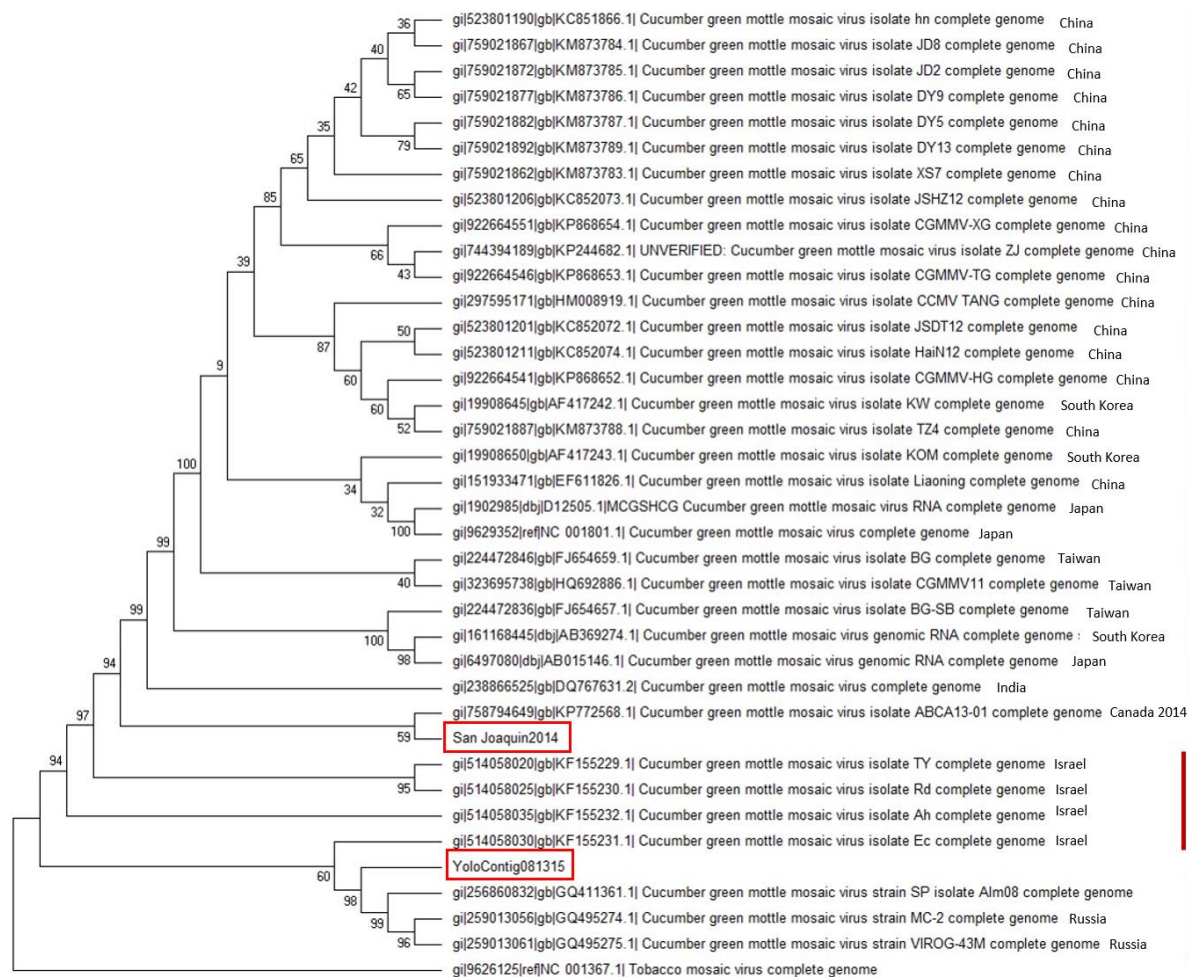


Figure 5. Phylogenetic comparison of the California 2013 and 2014 CGMMV genomic RNA sequences with those of other worldwide CGMMV isolates.

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